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(54) Title: METHOD FOR DIAGNOSIS OSTEOPENIA AND DETERMINING ITS SEVERITY

(57) Abstract

The present invention relates to a method of determining the severity of diseases such as osteopenia by measuring the concentrations of certain blood constituents and then calculating a bone density coefficient. The blood constituents required for diagnosing osteopenia according to the present invention are calcium, phosphate, estradiol, progesterone, total alkaline phosphatase and an alkaline phosphatase isoenzyme. A bone density coefficient is calculated using the blood concentrations of these blood constituents. The bone density coefficient can then be used to classify severity of osteopenia in the patient.

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METHOD FOR DIAGNOSING OSTEOPENIA AND DETERMINING ITS SEVERITY

Technical Field

The present invention relates to methods for diagnosing osteopenia. More particularly, the present invention relates to a method for diagnosing osteopenia in a human or animal and determining its severity and cause by measuring blood serum levels of specific, predetermined blood constituents and then calculating a severity index. Optionally, the age of the human or animal can be factored into the calculation.

Background of the Invention

The term "osteopenia" as used herein means any decrease in bone mass below the normal. The term "osteoporosis" as used herein means a specific form of generalized osteopenia characterized by a decrease in bone density, low bone mass, and microarchitectural deterioration of bone tissue. Osteoporosis can lead to enhanced bone fragility and a consequent increase in fracture risk. Osteoporosis may be idiopathic or may occur secondary to other diseases.

Bone consists primarily of an extracellular matrix containing (by weight) approximately 35% organic and 65%

inorganic components. The cells of bone represent a minor component of the bone constituents, yet they carry out a major portion of the function of the skeletal system. Bone cells help maintain serum calcium concentration within a narrow range to regulate mineral homeostasis while also being responsible for the continuous formation and resorption of the extracellular matrix, allowing response of the skeletal system to the mechanical forces resulting from physical activity.

The major organic component of the extracellular matrix is collagen. This protein has a rigid rodlike structure and is composed of three alpha chains held together in helical fashion by covalent and noncovalent forces. Multiple collagen molecules form fibrils, and these fibrils in turn are arranged in bundles or fibers. It is the bundles or fibers of collagen that can be seen in the light microscope as layers or linear arrays.

Non-collagen components of bone compose a very small portion of the organic matrix of the skeleton. This minor fraction consists of proteins, glycoproteins, mucopolysaccharides and lipids. Only a few of these components have been carefully identified and characterized. Two of the proteins that have been isolated and studied are osteonectin and osteocalcin. The synthesis of osteocalcin is vitamin K-dependent. This 6000 dalton protein contains the unique calcium-binding amino acid gamma-carboxyglutamic acid or Gla. Hence, osteocalcin has also been referred to in the literature as BGP, or bone Gla protein. Osteocalcin is synthesized by bone cells. Osteonectin is present in bone in the next highest concentration of non-collagen components. It is a protein with a molecular weight of 32,000 daltons that studies have implicated in the binding of calcium to collagen.

Calcium and phosphorus are the main components of the inorganic portion of the skeleton. Initially, calcium and phosphorus are deposited as amorphous salts but later undergo rearrangements into a crystalline structure that resembles hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). Several other ions,

including Na, K, Mg, and CO_3 in varying proportions, may be found in the skeletal hydroxyapatite. If there has been fluoride intake, there will also be F in the hydroxyapatite.

Although considerable effort has been expended in studying the mechanism of bone mineralization, it is not fully understood. Several theories have been suggested to explain all the available data. One theory is that calcium and phosphorus ions are present in the extracellular fluid in amounts exceeding the solubility product of [Ca] x [P]. These ions are kept from precipitating by inhibitors of calcification such as pyrophosphate. Because osteoblasts contain large amounts of alkaline phosphatase, it has been speculated that the activity of this enzyme facilitates mineralization by cleaving the phosphate groups, thus altering the Ca:P ratio in the sites of calcification.

Osteoporosis is the result of the gradual depletion of the inorganic portion of the skeleton and can be caused by any number of factors. Osteoporosis can affect men, women and even children. The following is a partial list of some of the categories of individuals at risk for developing osteoporosis:

Post-menopausal women
Cigarette smokers
Heavy users of alcohol
Users of a variety of drugs, such as steroids
Female runners and ballet dancers
Male marathoners consuming two few calories
Bulimics and anorexics
Teenagers on junk food
People allergic to dairy products
People affected with cancer
Fair and slim women
All men and women over the age of 65.

In addition to being female, the three most significant risk factors are poor diet, lack of exercise, and being postmenopausal. Other risk factors which are associated with osteoporosis include a Caucasian or Oriental background, a fair complexion, and a family history of osteoporosis.

Osteoporosis poses a major health problem in the United States, not only for those persons who are already affected but for individuals whose diet, life style, and body build make them more likely to develop osteoporosis as they age. Postmenopausal osteoporosis is a common disorder that results in substantial morbidity and mortality. As many as 25 million American women suffer from osteoporosis.

Osteopenia encompasses a group of diseases with diverse etiologies typified by reduction in bone mass per unit volume to a level below that which is necessary for adequate mechanical support function. Primary osteoporosis is an age related disorder that is particularly common in women and is characterized by decreased bone mass in the absence of other recognizable causes. However, osteoporosis occurs in both men and women. In women it is recognized usually at the 5th or 6th decade following menopause. In men osteoporosis is often recognized around their 6th or 7th decade. As the osteopenia progresses, both the number and thickness of the trabecular units decrease causing fragility of bone and an increased risk of fractures. Osteoporosis is responsible for approximately 250,000 femoral neck fractures annually in the United States, and these fractures are associated with a 20% mortality rate within 6 months. The risk is particularly high among the elderly, who also tend to lose bone as a result of the aging process. Wu K., et al., "Bone Resorption Rates in Physiological, Senile, and Postmenopausal Osteoporosis." *J. Lab Clin. Med.*, Vol. 69, pg. 810, (1967).

The onset of osteoporosis may be insidious or sudden, following trauma. The most common complaint associated with osteoporosis is back pain. This pain may be referred

along the course of involved nerves, particularly down the posterior aspect of one or both legs. Eventually, the pain may spread to the pelvis, the thorax, and the shoulders. In the spine, the vertebrae can compress, and the back can take on a "bent" appearance. Conditions such as kyphosis (humpback) or scoliosis may occur. If the spine becomes deformed, other body parts can be affected as well. For example, the ribs can be pushed against the pelvis, or the stomach can be pushed into the pelvis. In addition to spinal problems, osteoporosis can also lead to fractures of the hip, wrist, and ribs. These fractures can occur with only slight "trauma" and sometimes can occur with no trauma at all. Mazess B., et al., "Bone Density of the Radius, Spine, and Proximal Femur in Osteroporosis," *J. of Bone and Mineral Research*, Vol. 3, pgs. 13-18, (1988); Riggs B. L., et al., "Involutional Osteoporosis", *New Engl. J. Med.*, Vol. 314, pgs. 1676-1686, (1986). The changes associated with osteoporosis are gradual so osteoporosis is often not detected in its early stages.

It is estimated from a survey of medical clinics that of those individuals living to age 85, 32% of the women and 17% of men will fracture hips, weakened by osteoporosis. In addition to the pain and suffering caused by these fractures, the monetary cost is great, accounting for well over 3.8 billion dollars per year in treatment for fractures of osteoporosis. Moreover, six to eight months following hip fracture about 50% of the osteoporotic patients are in need of assistance with activities of daily living and about 25% require nursing home care. Only about 25% of the patients fully recover. In view of the costs associated with osteoporosis, as measured in dollars and in human suffering, osteoporosis has increasingly been perceived as a serious and disabling disease, warranting substantial involvement on the part of clinical investigators, governmental agencies, and pharmaceutical industries to develop and evaluate potential treatments and early detection techniques.

Current standard diagnostic techniques are not effective for early detection of osteoporosis. Changes seen in osteoporosis are very gradual and osteoporosis is often not detected in its early stages because bone mass must be decreased by about 30% to 40% before it is apparent using standard x-ray diagnostic techniques. Preventing osteoporosis by detecting early bone loss is far better than identifying the disease at relatively advanced stages and subsequently attempting to prevent its progression. Once major deterioration has occurred and gaps exist between the ends of fractured trabecular, no current treatment can be expected to restore the lost bone. Thus, therapeutic efforts must be directed toward prevention and early recognition of the progressive disease so treatment can be instituted before essentially irreversible structural damage ensues. Cummings S.R., et al., "Should Perimenopausal Women Be Screened for Osteoporosis?", *Ann. Int. Med.*, Vol. 104, pgs. 745-751, (1986); Courpron P., "Bone Tissue Mechanisms Underlying Osteoporosis," *Orthop. Clin. North Am.*, Vol. 12, pg. 513, (1981); Frost H. M., "Mechanical Determinants of Bone Modeling," *J. Metabol. Bone. Dis. Rel. Res.*, Vol. 4, pg. 217, (1983).

The radiographic manifestations of osteoporosis reflect the deficiency of the organic matrix and parallel the gross pathologic findings. The cortices are thin and the trabeculae fine and sparse; the skeletal structures are therefore more radiolucent than normal. The disease process eventually affects almost all of the skeletal structures. The principal areas of demineralization are the spine and pelvis, especially in the femoral neck and head. Demineralization is less marked in the skull and extremities. Although useful to detect breaks in the bone, ordinary x-rays are not sensitive enough to detect osteoporosis until a large amount of bone tissue has already been lost, generally from 25% to 40%. By the time

osteoporosis can be identified by x-ray techniques, the condition is advanced.

An early decrease in bone mass can be measured by non-invasive assessment of the skeleton by four widely available methods, including single photon absorptometry, dual photon absorptometry, dual-energy x-ray absorptometry, and quantitative computed tomography.

A device called a single-photon absorptometer (SPA) is used to measure bone mineral content, primarily in the forearm and wrist. The heel bone can also measured using SPA because the heel bone is thought to be a predictor of bone loss in the spine. SPA measures primarily cortical bone, which is also affected by osteoporosis, though not to the same extent as trabecular bone.

The technique of dual-photon absorptometry (DPA) provides a measurement of the total cortical and trabecular mineral content of the hip and spine. DPA uses less radiation than conventional x-rays; but, a scan of the spine using DPA still exposes the patient to approximately one tenth of the radiation that results from a routine chest x-ray.

Dual-energy x-ray absorptometry (DXA) provides a measurement of the amount of bone tissue in the hip and spine. This technique is now used routinely because it is faster than DPA.

Unlike DPA and DXA, quantitative computed tomography, more commonly called a CAT scan, can measure the density of either bone or just the trabecular portion. CAT scans unfortunately expose patients to higher doses of radiation than any of the other techniques.

Radiographic absorptometry (RA) is a method for non-invasive measurement of bone mineral x-rays of the hand. Radiographs, taken with a standard x-ray machine, are sent to a central laboratory for computer-controlled analysis.

One of the core problems with all of these current methodologies for determining whether a patient is suffering

from osteoporosis is that the procedures do not give any information about the underlying cause of the osteoporosis. For example, a common cause of postmenopausal osteoporosis is an estrogen deficit, which x-ray techniques cannot measure.

Another key problem in advancing the medical management of the osteopenic patient is that all the current methodologies require expensive, sophisticated medical instrumentation to perform the bone density measurements. Additionally, patients must be exposed to x-rays. This makes a general screening of high risk populations impractical due to the expense and unavailability of the necessary instrumentation to the average clinic.

The present invention generates a new level of interest in screening individuals who are at risk for osteoporosis because this novel diagnostic procedure provides information about the underlying cause of the osteopenia. The present invention also provides a simple, inexpensive, and rapid method of determining bone density using serum blood levels of specific, predetermined blood constituents.

In the clinical syndrome of osteoporosis, the reduction in bone mass can be attributed to osteopenia because of dietary deficiency or absorption interference of proteins or Vitamin C. It can also be the result of a deficient stress stimulus. Osteopenia can also be caused by osteomalacia, a failure of proper mineralization of osteoid resulting from bone calcium or phosphorus deficiency or both. It can also be caused by insufficient absorption from the intestine due to lack of calcium or a resistance to the action of Vitamin D due to failure of its conversion to the biologically active forms, 25 hydroxychole-calciferol and 1.25 dihydroxychole-calciferol, formed by the liver and kidney, respectively. In addition, it may well be caused by an abnormal rate of osteolysis due to parathyroid hormone stimulation of osteoclastic activity (osteocasts are hematopoitic in origin, arising from the migration of monocytes to bone). In reality, most cases of

osteoporosis, when carefully analyzed, reveal evidence of the causes of osteopenia.

Chemical analysis of blood may reveal calcium, phosphorus, and alkaline phosphatase within the normal range. However, an isoenzyme of alkaline phosphatase may be significantly increased. Increased bone resorption seen in osteoporotic patients, which occurs as a result of the action of osteoclasts, usually involves the dissolution of both minerals and organic matrix eventually leading to increased excretion of urinary hydroxyproline. Serum estradiol which is secreted almost entirely by the ovary is significantly decreased in these patients. This observation is further corroborated where it has been demonstrated that exogenous estrogen therapy in perimenopausal women does delay the onset of post menopausal osteopenia. Most experts agree that estrogen appears to decrease bone resorption. Weiss N. S., et al., "Decreased Risk of Fractures of the Hip and Lower Forearm with Postmenopausal Use of Estrogen," *N. Engl. J. Med.*, Vol. 303, pgs. 1195-1198 (1980); Ettinger B., et al., "Long Term Estrogen Replacement Therapy Prevents Bone Loss and Fractures," *Ann. Intern. Med.*, Vol. 102, pgs. 319-324, (1985).

It is believed that blood levels of calcium are maintained without increasing calcium loss from the bone as long as there are normal amounts of estrogen and parathyroid hormone present. It is reasonably excepted that estrogen antagonizes the effect of parathyroid hormone. Estrogen deficiency, as seen in peri- and postmenopausal women, results in an increase in the sensitivity of bone to parathyroid hormone. This antagonistic relationship eventually leads to an increase in the resorption of the bone and contributes to the development of Type I Osteoporosis. Type II Osteoporosis is characterized by reduced calcium absorption, which in turn results in increased secretion of parathyroid hormone. Type II Osteoporosis occurs in older individuals and is associated with wedge

fractures of the spine and fractures of the hip. A theory recently advanced by Barnhill, et al. suggests that evidence of decreased serum estradiol and increased lymphocyte alkaline phosphatase, represents an activated immune system in osteopenic postmenopausal women. This finding suggests that "uncovered estrogen receptors" may induce an immune reaction which is responsible for one form of osteoporosis. Barnhill S.D., et al., "Osteoporosis: A Possible Autoimmune Etiology," *Ann. of Clin. Lab. Sci.*, Vol. 17, pgs. 255-256, (1987).

In 1987, Barnhill, et al. proposed an autoimmune etiology for some forms of osteopenia. In that publication, Barnhill, et al. showed that lymphocyte-derived alkaline phosphatase was present in the blood of 90% of severely osteopenic women. See Barnhill, et al., "Osteoporosis: A possible Autoimmune Etiology", *Ann. of Clin. Lab. Sci.*, Vol. 17, pgs. 255-256, (1987)) The concept of a lymphocyte-derived alkaline phosphatase is further described by Griffiths, et al. See Griffiths, J., et al., "Separation and Identification of Alkaline Phosphatase Isoenzymes and Isoforms in Serum of Healthy Persons by Isoelectric Focusing", *Clin Chem*, Vol. 32, pgs. 2171-2177, (1987).

From the research perspective, osteoporosis can be classified as either primary or secondary to another disease. Primary osteoporosis is further classified as juvenile, idiopathic, postmenopausal (Type I) and involutional (Type II). It is now understood that accelerated bone loss occurs with cessation of menstruation at the time of menopause and in women who have amenorrhea as a result of prolactin producing pituitary tumor, anorexia nervosa, or intense long-distance running associated with undernourishment. These situations are all accompanied by estrogen deficiency which is likely to be a major determinant of the accelerated bone loss. Bone loss also occurs when estrogen therapy is withdrawn.

The National Institutes of Health 1984 Consensus Conference stimulated those interested in bone density with its recommendations for "defining persons at risk, and developing safe, effective and low cost strategies for fracture protection." See, Office of Medical Applications of Research National Institutes of Health, "Osteoporosis: consensus conference," *JAMA*, Vol. 252, pgs. 799-802, (1984). Since 1984, there has been a dramatic change in the way physicians view osteopenia. With the development and popularization of highly sensitive techniques such as DPA and quantitative computed tomography, physicians are now capable of measuring the density of the proximal femur and the lumbar vertebrae. (For a general review of the methods currently available to measure bone density, see Avioli, L.V., ed., "Metabolic Bone Disease and Clinically Related Disorders", W.B. Saunders Company (1990)).

Thus, there is new interest in screening the individuals who are at high risk for osteopenia. The current methods are relatively expensive and require specialized equipment to properly evaluate a patient. In addition, the current methods of measuring bone density do not give any information on the underlying cause of the osteopenia. What is needed is a simple, inexpensive, and rapid method of determining bone density. In addition, the method should also give information about the underlying cause of the osteopenia.

Summary of the Invention

The present invention includes a method and system for diagnosing and determining the severity and underlying cause of osteopenia using blood concentrations of a specific, predetermined set of blood constituents. The method comprises the steps of (1) measuring the severity of the disease in a set of humans or animals with varying severity of disease by a standard method, (2) assigning the severity of disease a numerical value on a severity scale, the scale being from no

disease to severe disease, (3) measuring the blood concentrations of the predetermined set of blood constituents in the set of humans or animals with varying severity of the disease, and then (4) calculating a numerical relationship between the set of blood concentrations and the severity of disease.

The present invention also comprises a simple and rapid method of determining severity of osteopenia in a specific patient. In a preferred embodiment, the method comprises determining the serum level of the following serum constituents: calcium, phosphate, total alkaline phosphatase, an alkaline phosphatase isoenzyme, estradiol, and progesterone. The alkaline phosphatase isoenzyme is preferable t-lymphocyte derived alkaline phosphatase or blood, liver or intestinal alkaline phosphatase isoenzyme. The results of these tests are then introduced into an algorithm. Optionally, the age of the patient may also be factored into the equation. The bone density coefficient that is calculated by the algorithm correlates to a very high degree to bone density as measured by standard methods, such as radiographic absorptometry, quantitative computed tomography, dual photon absorptometry and direct measurement of bone density. The bone density coefficient that is measured is then compared to an osteopenic severity scale.

Using the six serum constituent concentrations, the present invention can be used to determine the osteopenic state of a patient as well as give an indication of the underlying cause of the osteopenia. The present invention can be correlated to any method of measuring bone density simply by recalculating the coefficients in the algorithm using a multiple linear regression analysis.

Accordingly, it is an object of the present invention is to provide a method for determining the severity of a disease using the blood concentrations of a predetermined set of blood constituents.

Another object of the present invention is to provide a simple and rapid chemical test for diagnosing osteoporosis.

Another object of the present invention is to provide a test for osteoporosis which will also give information as to the underlying cause of the osteopenic condition.

Another object of the present invention is to provide a diagnostic test for osteoporosis which can be used to screen large numbers of individuals.

Yet another object of the present invention is to provide a method for diagnosing osteoporosis and determining the underlying cause of the osteopenia without having to subject the patient to radiation.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiment and the appended claims.

Brief Description of the Figures

Figure 1 is a linear regression analysis of the correlation between certain blood tests and DPA.

Figure 2 illustrates the discrepancies between the DPA measurements and the measurements obtained using the method of the present invention for individual subjects.

Figure 3 illustrates the correlation of the results obtained using the present invention and DPA.

Figure 4 demonstrates the normality of values predicted by the present invention.

Figure 5 demonstrates the normality of residuals (differences between DPA measurements and values predicted according to the method of the present invention), as evidenced by the arrangement of points along a diagonal straight line.

Figure 6 demonstrates that the variance of residuals.

Figure 7 demonstrates the independence of residuals.

Figure 8 demonstrates that virtually all observed DPA

measurements are predicted by the method of the present invention.

Detailed Description

The present invention relates to a method for diagnosing diseases in humans or animals using blood concentrations of specific, predetermined blood constituents. More specifically, the present invention relates to a method for detecting the occurrence of osteopenia, diagnosing osteoporosis and determining its severity and underlying cause. The present invention also facilitates the periodic monitoring of specific physiological functions which may indicate the onset of osteopenia and correlates to bone mineral density measurements determined by various standard methods.

In practicing one aspect of the present invention, the severity of disease in a set of humans or animals with varying severity of disease is measured by a standard method or methods. The measurement is then assigned a numerical value corresponding to a severity scale. The scale ranges from humans or animals with no disease, to humans or animals with severe disease. The scale is preferably a numerical scale. For example, one could assign a value which corresponds to normal or slight disease, another value which corresponds to moderate disease and a third value which corresponds to severe disease.

The concentration of a predetermined set of blood constituents in the set of humans or animals with varying severity of disease is then determined. According to the present invention; it is preferable to measure the blood constituents in the same set of humans or animals in which the severity of disease was measured by the conventional method or methods.

After determining the blood concentrations of the predetermined set of blood constituents in the set of humans or animals with varying severity of the disease, a mathematical

manipulation is performed in which the numerical value obtained using the conventional diagnosis is set to equal the result of a mathematical model utilizing the set of blood constituent measurements. For example, the relationship can be made using a multiple linear regression analysis. It is to be understood that other mathematical models could be used to determine a correlation between the serum constituent concentrations and a standard method of determining severity of the disease. The concept of using mathematical models to determine the relationship or correlation is considered to be part of the present invention. Standard statistical analyses that are well-known to those of ordinary skill in the art are then performed to determined the confidence level of the correlation between the diagnosis by conventional means and the set of blood constituents. These statistical analyses include chi-square tests.

An example of practicing one embodiment of the present invention is a method for diagnosing osteopenia in a human or animal. The method preferably utilizes six blood constituents. These constituents are calcium, phosphate, total alkaline phosphatase, an alkaline phosphatase isoenzyme, estradiol, and progesterone. The alkaline phosphatase isoenzymes preferred for practicing the present invention include lymphocyte-derived alkaline phosphatase isoenzyme and bone, liver or intestinal alkaline phosphatase isoenzymes. The present invention includes calculating a bone density quotient using the aforementioned six blood constituents by entering the values for the tests into an algorithm that is calculated using a multiple linear regression analysis. Optionally, the age of the patient may also be incorporated into the equation. The bone density coefficient derived from the algorithm allows one to diagnose the osteopenic state of the patient, including the severity of the disease.

In addition to diagnosing the osteopenic state of the human or animal, an indication of the underlying cause of the

osteopenia can be determined using the present invention. For example, by practicing the present invention as described herein, one can determine whether the osteopenia in a human or animal is caused by post-menopausal lack of estrogen or is caused by some other condition, such as cancer. This allows the attending physician to be better able to prescribe the appropriate treatment for the osteopenia.

Five of the serum tests that are used in the present invention are tests that are commonly performed by clinical laboratories. The test for t-lymphocyte derived alkaline phosphatase is experimental only; however, the test for blood, liver and intestinal alkaline phosphotase isoenzymes are also known. The type of test used to determine the six serum constituents is not critical to the present invention as long as the tests give accurate blood concentrations of the constituents being measured.

Calcium & Phosphorus:

Maintenance of calcium and phosphorus homeostasis involves the participation of 3 major organs: the small intestine, the kidney, and the skeleton, and is regulated by various hormones. Calcium enters the body through the diet and is absorbed into the circulation from the small intestine. Calcium absorption occurs by two processes, active transport and passive transport. Approximately 98% of the calcium and 85% of the phosphorus in the adult is present in the skeleton primarily as hydroxyapatite, which is a crystal lattice compound of calcium, phosphorus, and hydroxide. Remaining calcium is present in extra cellular fluid, some types of tissue, and skeletal muscle. Phosphorus is combined with lipids, proteins, carbohydrates, and other organic substances. Of critical importance to calcium homeostasis is the fact that less than 1% of total skeletal reservoir of calcium is rapidly exchangeable with extracellular fluid. In addition to its obvious importance in skeletal mineralization, it is also vital for blood coagulation, neuromolecular condition, maintenance of normal tone and excitability of skeletal and cardiac muscle, and preservation of all membrane integrity and permeability, particularly in terms of sodium and potassium exchange.

Free or ionized calcium accounts for 50% of total calcium. About 5% of total calcium is complexed with a variety of anions, particularly phosphate and citrate. The remaining 45% of calcium is bound to plasma proteins. Both ionized calcium and calcium complexes are freely dialyzable. Acidotic and alkalotic conditions adversely affect the ionized calcium level in the blood. In metabolic bone disease such as hyper- or hypoparathyroidism, Pagets of bone, Vitamin D deficiency, and renal osteodystrophy, calcium and/or phosphorus levels are significantly altered.

Serum Calcium Determination:

In one method of practicing the present invention, the procedure for the determination of calcium is based on the interaction of the chromogenic agent o-cresolphthalein complexone (Sigma Diagnostics Calcium Agent, Sigma Chemical Co., St. Louis, MO) which complexes with calcium cation in an alkaline medium to form a purple colored complex which has absorbance maximum at 575 nm. The intensity of the color measured at 575 nm is directly proportional to the calcium concentration in the given sample. The o-cresolphthalein complexone contains 8-hydroxyquinoline which prevents interference from magnesium ions. *Robertson, W.G.*, et al., "Calcium Measurements In Serum and Plasma - Total and Inonized," *Crit. Rev. Clin. Lab. Sci.*, Vol. 11, pg. 271, (1979); Sigma Diagnostics Product Insert: Calcium, Procedure No. 587, Reissued May 1989. The calcium level in serum is in the range of about 9.2 to 11.0 mg/dl which is composed of 3 distinct fractions.

Specimen Collection:

In one method of practicing the present invention, serum or heparinized plasma are suitable for the test. Anticoagulants other than heparin should not be used.

Procedure:

Sigma Diagnostics Calcium Reagent is used with Abbott Spectrum High Performance Diagnostic System for the quantitation of calcium in serum. It is generally considered good laboratory practice to run a calibration, linearity, and a quality control prior to the determination of patient samples. Calcium levels up to 16 mgs % can be measured by this method.

Serum Phosphorus Determination:

In one method of practicing the present invention, the procedure for the determination of serum phosphorus is based on the interaction of inorganic phosphorus with ammonium molybdate in the presence of sulfuric acid. Barnhill S., et al., "Osteoporosis: A Possible Autoimmune Etiology," *Ann. of Clin. Lab. Sci.*, Vol. 17, pg. 255, (1987). This reaction produces unreduced phosphomolybdate complex. The absorbance of this complex at 340 nm is directly proportional to the inorganic phosphorus present in the given sample. (Sigma Diagnostics Product Insert: Phosphorus, Inorganic, Procedure No. 360-UV. Previous Revision December 1985. Reissued July (1988)). Most of the phosphorus in extracellular fluid is inorganic and in the range of 2.4 to 4.7 mg/dl.

Specimen Collection:

Serum or heparinized plasma are preferred for the test. Anticoagulants other than heparin should not be used.

Procedure:

Sigma Chemical Company's phosphorus inorganic reagent can be used with the Abbott Spectrum High Performance Diagnostic System for qualification of the inorganic phosphorus in serum. It is customary to perform calibration, linearity, and quality control studies prior to the determination of test samples. Phosphorus levels up to 12 mgs % can be measured by this procedure.

Total Alkaline Phosphatase:

The demonstration that bone is rich in alkaline phosphatase (ALP) and that normal serum contains the same or a similar enzyme led to the study of serum ALP levels in patients with disease of bone. See, Courpron P., "Bone Tissue Mechanisms Underlying Osteoporosis," *Orthop. Clin. North Am.*, Vol. 12, page 513, (1981). This is especially true in

osteitis deformans, hyperparathyroidism and bone neoplasm. ALP is also elevated in hepatic disease; however, this can be distinguished by other corroborative laboratory procedures and clinical features. In some situations, as in osteoporosis, ALP *per se* may not be above the reference range; however, the isoenzyme of ALP is increased. It is well understood that total serum ALP in normal subjects consists of isoenzymes contributed from liver, bone, renal, pulmonary, placental and intestinal sources, among others. The isoenzymes and isoforms of various tissue origins can be further separated and visualized by an Isoelectric Focusing Technique. Estimation of total serum ALP by isoelectric focusing techniques offers great potential in the investigation of metabolic bone diseases.

Serum Alkaline Phosphatase Determination:

In one method of practicing the present invention, serum ALP activity can be measured using various phosphate esters as substrates. Sigma Chemical Company's alkaline phosphatase reagent measures serum ALP activity by a kinetic method. The reagent for the test contains p-nitrophenyl phosphate, carbonate buffer, magnesium ions and mannitol. Mannitol present in the reagent acts as a phosphate acceptor during the enzyme reaction. McComb R.B., et al., Alkaline Phosphatase. Plenum Press, New York, (1979); Gundbery, M., Alkaline Phosphatase and Osteocalcin, Primer on Metabolic Bone Diseases and Disorders of Mineral Metabolism 1st Edition, Published by Am. Soc. For Bone and Mineral Res. Editor-Murry J. Favus, pgs. 74-76, (1990); Sigma Diagnostics Product Insert: Alkaline Phosphatase (ALP), Procedure No. 245, Reissued March 1989; Kaplan T.A.,et al., Clinical Chemistry. St. Louis, e.v. Mosby Company, (1987).

Specimen Collection:

Preferably, serum or heparinized plasma is used for the total alkaline phosphatase and alkaline phosphatase isoenzyme

determination tests. EDTA, oxalate, citrate and fluoride are inhibitors of ALP and are not suitable anticoagulants.

Procedure:

Alkaline phosphatase (ALP) diagnostic reagent manufactured by Sigma Chemical Company is used with the Abbott Spectrum High Performance Diagnostic System for the quantitation of inorganic phosphorus in the serum. Serum ALP hydrolyzes p-nitrophenyl phosphate to p-nitrophenol and inorganic phosphate. The hydrolysis occurs at alkaline pH and the absorbence is directly proportional to the ALP activity of the serum sample. As is the usual practice, calibration, linearity and quality control assays should be performed prior to the determination of test samples.

ALP levels up to 1200 U/L can be measured by this procedure. Normal ranges are:

Infants	50-165	(U/L)
Adult	20-70	(U/L)
Child	20-150	(U/L)
60 years	30-75	(U/L)

Alkaline Phosphatase Isoenzyme:

Alkaline phosphatase isoenzyme levels have been primarily used to aid in the differential diagnosis of liver and bone disorders and also to indicate placental growth patterns. Isoelectric focusing of alkaline phosphatase isoenzyme on a precast gel gives 12 discernible bands of different organ and tissue origin. Of these 12 Bands, Band 10, appearing at 4.73 isoelectric point, is of placental origin. It is released by placental syncytiotrophoblast and/or macrophage, and is T-lymphocyte mediated. In the alternative, samples for ALP isoenzyme can be analyzed by electrophoresis methods which identify mainly liver, bone, and intestinal isoenzymes. The Ciba Corning Alkaline Phosphatase Isoenzyme (ALP) System

may be used for the qualitative and quantitative determination of ALP isoenzymes in human serum by electrophoresis.

The liver isoenzyme is the most frequently encountered alkaline phosphatase isoenzyme in pathologically increased serum alkaline phosphatase activity. Increased liver isoenzyme is encountered in a variety of liver and hepatobiliary diseases. These include hepatic cirrhosis, primary biliary cirrhosis, congestive cirrhosis, and hepatic carcinoma. Elevation of the liver isoenzyme is also a sensitive indicator of cholestasis and liver infiltration.

Another of the alkaline phosphatase isoenzymes that can be used in practicing the present invention is lymphocyte-derived alkaline phosphatase. The preferred method of measuring this isoenzyme is by isoelectric focusing electrophoresis. A commercially available isoelectric focusing apparatus that is capable of measuring alkaline phosphatase isoenzymes is made by IsoLab, Inc. (Resolve®-ALP, IsoLab, Inc., Akron, Ohio). On the Resolve®-ALP isoelectric focusing apparatus, the alkaline phosphatase isoenzyme that is used in the present invention resolves as band 10 in the electrophoretic pattern. In the preferred bone density algorithm defined hereinbelow, the value for the band 10 alkaline phosphatase isoenzyme was assigned 0 if the band were missing or very weak, and 1 if the band were present. The electrophoresis gels can also be scanned with a densitometer and more quantitative values can be assigned to the alkaline phosphatase isoenzyme concentration. As used herein, the band 10 alkaline phosphatase isoenzyme shall be designated lymphocyte-derived alkaline phosphatase isoenzyme, or I-Alkp.

It is to be understood that other methods of measuring I-Alkp could be used. These methods include, but are not limited to, enzyme-linked immunoassay techniques (ELISA), radioimmunoassay techniques, affinity columns, and isoelectric focusing columns. It is important to note that measurement of

band 10 on the Resolve®-ALP apparatus does not correlate at all with osteopenia in general. There are many other abnormal conditions which result in a higher than normal band 10 lymphocyte-derived alkaline phosphatase isoenzyme (see *Barnhill, et al.* and *Griffith, et al.*, supra.).

A number of pathological conditions can lead to an elevated level of bone alkaline phosphatase isoenzyme. The highest levels of bone isoenzymes are usually found in Pagets disease. Increased levels are also encountered in rickets, bone cancer, osteomacacia and celiac sprue. Renal disorders can also result in increased levels. These include renal failure, primary hyperparathyroidism, secondary hyperparathyroidism induced by long term hemodialysis and **malabsorption.**

Increased levels of intestinal alkaline phosphatase are encountered in a variety of diseases of the digestive tract. These include intestinal infection and ulcerative lesions of the stomach, duodenum, small intestine and colon.

Procedure:

In the preferred method of practicing the present invention, the alkaline phosphatase isoenzymes are separated by electrophoresis in a buffered agarose system. After electrophoresis the isoenzymes are detected by incubating the gel with a fluorescent compound such as 4-methylumbelliferyl phosphate. The fluorescense formed during the interaction is quantitated using a Ciba-corning 710 densitometer at 385 nm. Total alkaline phosphatase activity of the patient is required to interpret the liver, bone and intestinal ALP isoenzymes.

Estrogen and Progesterone:

The estrogens are steroids that have a ring containing three unsaturated double bonds. The ovary, as well as the testes and adrenal gland, has the capacity to synthesize estrogens from androgens, androstenedione and testosterone. During the follicular phase of the menstrual cycle, ovarian

secretion represents only one third of total estrogen production. In contrast to estradiol, which is secreted almost entirely by the ovary, most estrone is derived from peripheral conversion of androstenedione and from estradiol metabolism. During menopause, estradiol concentrations steadily decrease to approximately 15 percent of premenopausal levels. In healthy postmenopausal women, the ovaries do not secrete significant quantities of estrogens, and virtually all estrogen produced is from peripheral conversion of androstenedione made by the adrenal. Estradiol is present in the serum as follows:

Follicular Phase	
Early	30-100 ng/L
Late	100-400 ng/L
Luteal Phase	50-150 ng/L
Postmenapausal	<20 ng/L

In menstruating females, progesterone is secreted mainly by the corpus luteum of the ovary. It is partially responsible for cyclic changes in the endometrium that are necessary for attachment and growth of an embryo. Progesterone levels are low prior to the mid-cycle gonadotropin surge. Shortly after the gonadotropin surge, they begin to rise rapidly, reaching peak levels during the middle of the luteal phase. Thereafter, a progressive fall occurs with barely detectable progesterone levels prior to menses.

Although progesterone in large amounts produces a negative feedback on gonadotropin secretion, it is not the major component in the negative feedback system of ovarian steroids. Function of the corpus luteum can be assessed by measuring serum progesterone concentration. Progesterone is present in the serum as follows:

Follicular Phase	0.1 - 1.5 ng/L
Luteal Phase	2.5 - 28.1 ng/L
Mid-Luteal Phase	5.7 - 28.1 ng
Over 60 Years	0.0 - 0.2 ng/L

Procedure:

In a preferred method of practicing the present invention, progesterone measurements can be obtained by a radioimmuno-assay method using antibody coated tubes (Diagnostic Products, Los Angles, California). Estradiol measurements can be performed by a Microparticle Enzyme Immunoassay (IMX), available from Abbott Diagnostics, Abbott Park, IL.

Bone Mineral Density (BMD) Measurements:

The conventional methods of diagnosing osteopenia which may be used when practicing the present invention are are outlined below:

Dual-Photon absorptometry (DPA) is widely used to assess bone mineral content and bone mineral density. Johnston Conrad C, et al., "Clinical Use of Bone Densitometry", *New Eng. J. of Med.*, Vol. 324, pgs. 1105-1109, (1991). DPA uses transmission scanning with photons from a radioisotope source, such as 153 Gd, that emits two energy peaks, thus allowing bone density to be measured independent of soft tissue. DPA measurements are performed on lumbar spine (L1-L4) and femur (femoral neck, Ward's Triangle and trochanter) and the average determined separately. The overall average of both hip and spine can also be determined. BMD can be measured by DPA using ~ Gadolinium as the source (Lunar DP3, by Lunar Radiation Corporation, Madison, Wisconsin).

The present method employs transmission scanning using 44 and 100 KeV photon energies from a one Ci^{153}Gd source to allow computation of the mineral content of bone

independent of soft tissue thickness. Bone mineral density, expressed in g/cm2, is derived by dividing bone mineral content (BMC) by the projected area of the scanned bone. Peppler W.W., et al., "Total Body Bone Mineral and Lean Body Mass by Dual-Photon Absorptometry: 1. Theory and Measurement Procedure," *CALCIP, Issue Int.*, Vol. 33, pg. 353, (1981); Shipp C., et al., "Precision of Dual-Photon Absorptometry," *CALCIP, Issue Int.*, Vol. 42, pgs. 287-292, (1988). During spine scans, the detector moves in a rectilinear pattern at a rate of 5mm/sec and with scan lines 4.5 mm apart. The BMC and BMD are calculated in lumbar vertebrae 1 through 4 (including intervertebral discs) with a software version supplied by Lunar Radiation Corporation of Madison, Wisconsin.

During femur measurements, the scanner moves at a rate of 2.5 mm/sec and a step distance of 2.5 mm. The BMC and BMD of the neck, Ward's Triangle and trochanteric regions of the proximal femur are calculated using a femur software version supplied by Lunar Radiation Corporation. The femoral neck region of interest (ROI) is that band about 1.5 cm wide across the neck of the bone perpendicular to the neutral axis with the lowest density. Ward's Triangle is defined as a square ROI (about 1.5 x 1.5 cm) with the lowest density within the proximal femur region. Ward's Triangle is predominantly trabecular bone and contains the least amount of bone mineral within the neck region. Carter D.R., et al., "Relationship Between Loading History and Femoral Cancellous Bone Architecture," *J. Biomechanics*, Vol. 22, pgs. 231-244, (1989). In the proximal femur, the ROI is usually the area 1.5 cm wide across the entire femoral neck. Additional regions are defined by the software in the lower density Ward's Triangle region, and in the region of the greater trochanter. Bone loss in the proximal femur begins in the Ward's Triangle region and proceeds outward from there. (Brown D., et al., "Mechanical Property Distributions in the

Cancellous Bone of the Human Proximal Femur," *Act. Orthop. Scand.*, Vol. 51, pgs. 429-437, (1990). This makes the region an early indicator of bone loss, but the higher variance in measuring it, compared to the neck region, makes the latter zone, a better discriminator. However, Ward's Triangle is the lowest density area at the point where the neck and greater trochanter meet, a primary hip fracture site. This operational ROI may not correspond exactly to the anatomic Ward's Triangle region but does provide a repeatable measurement. The width of the neck ROI and the size of the Ward's Triangle ROI are actually proportional to the measured size of the femoral neck. It has been shown that the density of the Ward's Triangle area is substantially reduced in hip fracture patients compared with age matched controls. (Vose G. P., et al., "Femoral Neck Fracturing its Relationship to Radiographic Bone Density," *J. Gerontol.*, Vol. 20, pgs. 300-305, (1965).

DPA measurements have a precision of 1-3% and the scan can be completed in about 20 minutes. Bone density of vertebrae correlates well with risk for vertebral fracture, and bone density of areas in the proximal femur correlates well with risk for hip fracture. DPA has a low radiation dose (<10 mrem to skin, and 2 mrem to marrow). DPA has approximate sensitivity of spine and hip of 50% and 53%, respectively, at 95% specificity (i.e. % of fracture cases below 5th percentile). Moreover, DPA is not subject to systematic errors introduced by variable osteoid and variable marrow.

Bone density can also be measured by radiographic absorptometry. Radiographic absorptometry is a method of measuring bone density which is well known to those of ordinary skill in the art. Other methods of measuring bone density include quantitative computed tomography and direct measurement of bone density.

Direct measurement of bone density can be obtained by measuring the bone density in cadavers. Thus, another method

of determining the correlation between the blood concentrations of certain predetermined blood constituents and bone density is to measure the bone density of a set of cadavers from which blood concentrations of predetermined blood constituents are known. The multiple linear regression analysis can then be performed and a bone density coefficient relationship can easily be determined.

In correlating the bone density measurements to concentration of blood constituents, blood concentrations of calcium, phosphate, total alkaline phosphatase, an alkaline phosphatase isoenzyme estradiol, and progesterone are measured. Liver, bone and intestinal isoenzymes can be used. Band 10 alkaline phosphatase isoenzyme. A mathematical relationship between the concentrations of blood constituents and bone density, as measured by radiographic absorptometry or other standard method of measuring bone density, is determined by performing a multiple linear regression analysis with the following model:

Bone density coefficient $=b_0 + b_1$Ca $+ b_2$P $+ b_3$E2 $+ b_4$Pg $+b_5$Alkp $+ b_6$I-Alkp $+ b_7$(CaCa) $+ b_8$(PP) $+ b_9$(E2E2) $+ b_{10}$(Pg + Pg) $+ b_{11}$(AlkpAlkp) $+ b_{12}$(CaP) $+ b_{13}$(CaE2) $+ b_{14}$(CaPg) $+b_{15}$(CaAlkp) $+ b_{16}$(CaI-Alkp) $+ b_{17}$(PE2) $+ b_{18}$(PPg) $+ b_{19}$(PAlkp) $+ b_{20}$(PI-Alkp) $+ b_{21}$(E2Pg) $+ b_{22}$(E2Alkp) $+ b_{23}$(E2I-Alkp) $+ b_{24}$(PgAlkp) $+ b_{25}$(PgI-Alkp) $+ b_{26}$(AlkpI-Alkp)

where:

Ca = Serum concentration of calcium

P = Serum concentration of phosphorus

E2 = Serum concentration of estradiol

PG = Serum concentration of progesterone

Alkp = Serum concentration of total alkaline phosphatase

I-Alkp = Serum concentration of alkaline phosphatase isoenzyme.

Calculation of the mathematical model utilized the Systat® statistical package (Systat: Inc., Evanston, IL). The multiple linear regression analysis is an iterative process which calculates the correct coefficients so that the result of the algorithm using the blood concentrations correlates to a high degree with the result of the bone density measurement by radiographic absorptometry.

For diagnosing osteopenia, the general form of the preferred algorithm that is used in the present invention is as follows:

Bone density coefficient = $b_0 + b_1$Ca + b_2P + b_3E2 + b_4Pg + b_5Alkp + b_6I-Alkp + b_7(CaCa) + b_8(PP) + b_9(E2E2) + b_{10}(Pg + Pg) + b_{11}(AlkpAlkp) + b_{12}(CaP) + b_{13}(CaE2) + b_{14}(CaPg) + b_{15}(CaAlkp) + b_{16}(CaI-Alkp) + b_{17}(PE2) + b_{18}(PPg) + b_{19}(PAlkp) + b_{20}(PI-Alkp) + b_{21}(E2Pg) + b_{22}(E2Alkp) + b_{23}(E2I-Alkp) + b_{24}(PgAlkp) + b_{25}(PgI-Alkp) + b_{26}(AlkpI-Alkp)

where:

b_0 is a constant

Ca = Serum concentration of calcium

P = Serum concentration of phosphorus

E2 = Serum concentration of estradiol

PG = Serum concentration of progesterone

Alkp = Serum concentration of total alkaline phosphatase

I-Alkp = Serum concentration of alkaline phosphatase isoenzyme.

It is to be understood that concentration of I-Alkp can be replaced with serum concentration of liver, bone or intestinal derived alkaline phosphatase isoenzymes.

In practicing the present invention, the values obtained from the patient for the six blood constituent concentrations are inserted into the algorithm as indicated. The mathematical manipulation is performed. The resulting number is called a

bone density coefficient and is then placed in the severity scale. This results in a bone density probability quotient which correlates to a high degree to bone density as measured by radiographic methods.

As seen in the algorithm that is considered part of the present invention, there are a number of coefficients which are a part of the algorithm. It is to be understood that these coefficients can change if the bone density coefficient is correlated to a different method of determining bone density. Thus, if bone density is measured using dual photon absorptometry, and one wanted to correlate the bone density coefficient to the results of the dual photon absorptometry, the overall relationship of the tests in the algorithm would be the same or similar as disclosed herein but the coefficients could be different.

In its preferred embodiment, the bone density probability quotient is assigned to one of the following diagnostic categories:

Normal to mild osteopenia
Moderate osteopenia
Severe osteopenia

In addition, the menopausal status may be determined based on the results of the estradiol and progesterone levels into one of the following diagnostic categories:

Probable Pre-menopausal
Probable Peri-menopausal
Probable Post-menopausal

Thus, according to the present invention, using blood concentrations of certain blood constituents, one can not only diagnose bone density but can also obtain an indication of the underlying cause of the osteopenia.

This invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. To the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

Example I

In the following example, the bone density coefficient algorithm was correlated with bone density as measured by radiographic absorptometry. The coefficients were calculated by correlating the blood concentration of the indicated blood constituents in 27 patients to the results of bone density in those patients as measured by radiographic absorptometry. The bone density algorithm which results is as follows:

$$0 + (-8.955)(Ca) + (79.370)(P) + (34.076)(E2) + (-9.216)(Pg) + (-0.600)(Alkp) + (-57.855)(I\text{-}Alkp) + (0.926)(CaCa) + (-2.735)(PP) + (-0.272)(E2E2) + (-0.064)(PgPg) + (0.004)(AlkpAlkp) + (-4.029)(CaP) + (-3.156)(CaE2) + (1.172)(CaPg) + (-0.031)(CaAlkp) + (8.238)(CaI\text{-}Alkp) + (1.906)(PE2) + (-0.138)(PPg) + (-0.018)(PAlkp) + (-6.710)(PI\text{-}Alkp) + (0.193)(E2Pg) + (-0.048)(E2Alkp) + (-1.825)(E2I\text{-}Alkp)$$

wherein:

Ca = Serum concentration of calcium

P = Serum concentration of phosphorus

E2 = Serum concentration of estradiol

PG = Serum concentration of progesterone

Alkp = Serum concentration of total alkaline phosphatase

I-Alkp = Serum concentration of serum lymphycyte-derived alkaline phosphatase isoenzyme.

The bone density coefficient that is obtained from the bone density algorithm is assigned to one of three categories:

Group 1	normal to mild osteopenia
Group II	moderate osteopenia
Group III	severe osteopenia

Group III ≤ approximately 78.5 < Group II ≤ approximately 100 < Group I

It has been determined that the bone density coefficient calculated according to the present invention places patients in the same groups as radiographic absorptometry measurements for bone density. Thus, the present invention provides a safe, economical and accurate method for diagnosing osteopenia.

Bone density quotients derived practicing the present invention using the six biochemical serum constituents were found to strongly correlate with bone density measurements from radiographic absorptometry. It is to be understood that the preferred embodiment utilizes all six of the biochemical serum constituents listed hereinabove. However, if any five of the serum constituents are utilized and then included in the bone density algorithm, a bone density quotient is obtained which does not correlate as well as when six tests are used.

It is contemplated as part of the present invention that any five of the biochemical serum constituents can be used to determine the osteopenic state of a patient. For example, if serum calcium is removed from the bone density algorithm, then the algorithm predictive value for moderate or severe osteopenia is approximately 66% although the predictive value for normal to mild osteopenia is 100%. If progesterone is removed from the bone density algorithm, then the algorithm predictive value for moderate or severe osteopenia of

approximately 75% and a predictive value for normal to mild osteopenia of approximately 94%. If alkaline phosphatase is removed from the bone density algorithm, then the algorithm predictive value for moderate or severe osteopenia of approximately 83% and a predictive value for normal to mild osteopenia of approximately 85%. If estradiol is removed from the bone density algorithm, then the algorithm predictive value for moderate or severe osteopenia of approximately 67% and a predictive value for normal to mild osteopenia of approximately 85%. However, it has been determined that when all six tests are included in the bone density algorithm, the predictive value for moderate and severe osteopenia is 100% and the predictive value for normal to mild osteopenia is 100%. All of the predictive values may vary slightly with different patient populations.

Example II

The following serum constituents are measured in a 37 year old woman:

Calcium	9.5 mg/dL
Phosphate	5.0 mg/dL
Estradiol	2 pg/mL
Progesterone	0.2 ng/mL
Alkaline Phosphatase	80 U/L
Alkaline phosphatase isoenzyme	0 (negative)

The above blood chemistry values were used to calculate a bone density coefficient using the following algorithm:

$$0 + (-8.955)(Ca) + (79.370)(P) + (34.076)(E2) + (-9.216)(Pg) + (-0.600)(Alkp) + (-57.855)(I\text{-}Alkp) + (0.926)(CaCa) + (-2.735)(PP) + (-0.272)(E2E2) + (-0.064)(PgPg) + (0.004)(AlkpAlkp) + (-4.029)(CaP) + (-3.156)(CaE2) + (1.172)(CaPg) + (-0.031)(CaAlkp) + (8.238)(CaI\text{-}Alkp) +$$

(1.906)(PE2) + (-0.138)(PPg) + (-0.018)(PAlkp) + (-6.710)(PI-Alkp) + (0.193)(E2Pg) + (-0.048)(E2Alkp) + (-1.825)(E2I-Alkp)

where:

Ca = Serum concentration of calcium

P = Serum concentration of phosphorus

E2 = Serum concentration of estradiol

PG = Serum concentration of progesterone

Alkp = Serum concentration of total alkaline phosphatase

I-Alkp = Serum concentration of serum lymphycyte-derived alkaline phosphatase isoenzyme.

A bone density coefficient of 101.2 is calculated for this patient. This coefficient falls within Group I in the following severity scale:

Group III ≤ 78.5 < Group II ≤ 100 < Group I

Group I	normal to mild osteopenia
Group II	moderate osteopenia
Group III	severe osteopenia

This patient has normal to mild osteopenia. When the patient's bone density is measured by radiographic absorptometry, she is found to have normal bone density.

EXAMPLE III

The following serum constituents are measured in a 47 year old woman:

Calcium	9.5 mg/dL
Phosphate	5.0 mg/dL
Estradiol	2 pg/mL
Progesterone	0.2 ng/ml
Alkaline Phosphatase	80 U/L

Alkaline phosphatase isoenzyme .....1 (positive)

The values are inserted into the bone density algorithm recited in Example I and a bone density coefficient of 84.4 is calculated. This falls in Group II, indicating a moderate osteopenia. When the patient's bone density was measured by radiographic absorptometry, she was found to be moderately osteopenic.

EXAMPLE IV

The following serum constituents are measured in a 47 year old woman who has been diagnosed with breast cancer:

Calcium .....9.0 mg/dL
Phosphate.....4.0 mg/dL
Estradiol.....0 pg/mL
Progesterone .....0 ng/mL
Alkaline Phosphatase.....60 U/L
Alkaline phosphatase isoenzyme .....1 (positive)

The values are inserted into the bone density algorithm recited in Example I and a bone density coefficient of 69.9 is calculated. This falls in Group III indicating a severe osteopenia insured by another method.

EXAMPLE V

The following example shows the correlation between the method of the present invention and Dual Photon Absorptometry (DPA) as a tool for diagnosing and determining the severity of osteoporosis in a particular individual. 200 female subjects representing a cross section of ages and menopausal status were evaluated. These specific age groups were represented as follows:

Age	Number of Patients
25-35	26
36-45	25
46-55	33
56-65	30
66-75	34
76-85	25
85 and over	26

Each subject completed an Osteoporosis Data Questionnaire Form, which included detailed information on personal history, family history, gynecologic history, medical history, surgical history, and drug history, with special references to hormone treatment.

Three tubes of blood were collected from each subject via venipuncture. One tube was sent to Barnhill-MetPath Laboratories in Savannah, Georgia for analysis of serum calcium, phosphorous, and total alkaline phosphatase. The second tube was sent to Horus Therapeutics, Inc. in Savannah, Georgia for determination of lymphocyte alkaline phosphatase utilizing the Isoelectric Focusing Method. The third tube was sent to MetPath Laboratories in Teterboro, New Jersey for evaluation of estradiol and progesterone by immunoassay. Data from all three sources were then subjected to statistical analysis. At the time of the analysis of data, each subject's chart was critically reviewed for relevant clinical information.

The Bone Mineral Density (BMD) of each subject was also measured by Dual-Photon Absorptometry using ^{153} Gadolinium as a source (using Lunar DP3, Lunar Radiation Corporation, Madison, Wisconsin). DPA measurements were performed on the Lumbar Spine (L1-L4) and the hip, specifically the femoral neck, Ward's Triangle, and the trochanteric regions. BMD was measured in gm/cm^2 for each region noted, and the fracture risk was determined.

The BMD measurements were then age matched, and adjusted for sex, age, ethnic group, and weight. From the individual values obtained, average BMD for hip and spine was calculated. The overall average BMD for both hip and spine was also computed.

A mathematical algorithm correlating the results using the present invention with DPA was determined with the resultant data is shown in Figure 1.

Figure 2 illustrates the discrepancies between the DPA measurements and the measurements obtained using the method of the present invention for individual subjects. The amount of the discrepancy (the residual) for any patient is the vertical distance between the points for that patient on the DPA and the present invention lines.

Figure 3 illustrates the appropriateness of using the present invention as a prediction of DPA measurements. Note the absence of any tendency for points to be consistently above or consistently below the diagonal straight line, as greater DPA measurements are considered. Additionally, there is an absence of any tendency for points along any vertical line to be more widespread than are points along any other vertical line. Finally, note that the generally elongated shape formed by the points is consistent with a strong linear correlation between DPA and the present invention.

Figure 4 demonstrates the normality of values predicted by the present invention (the horizontal scale). The vertical scale shows the of the normalized scale (z) scores values determined according to the present invention as would be expected from the assumption of the present invention values forming a normally distributed collection of numbers.

The closer the dots are to the straight line, the greater is the consistency between what is observed about the present invention values and what is expected about them from the assumption of their forming a normally distributed collection of numbers.

Figure 5 demonstrates the normality of residuals (the difference between the observed DPA measurement and the value predicated by present invention), as evidenced by the arrangements of points along the diagonal straight line. The horizontal scale shows values of residuals The vertical scale shows the residuals' normalized scale (z) scores as would be expected from the assumption that the residuals form a normally distributed collection of numbers.

The closer the dots are to the straight line, the greater is the consistency between what is observed about the residuals and what is expected about them from the assumption of their forming a normally distributed collection of numbers.

Figure 6 demonstrates that the variance of residuals stays the same regardless of the value calculated according to the present invention involved in the residuals' computation. This homogeneity of variance appears in the uniform density of the dots in a rectangle formed by residuals between -0.17 and +0.17 and by the present invention between +0.4 and +1.0. Moreover, there is no discernable tendency for points along any vertical line to be more widespread than are points along any other vertical line.

Figure 7 demonstrates the independence of residuals and the values predicted by the present invention. The horizontal scales shows the values predicted by the present invention. The vertical scale shows the residual's studentized scale (t) scores.

When there is no relationship between values predicted by the present invention and the residuals involving those predicted values (that is, when there is independence between residuals and predicted present invention values) the following criteria should be met:

1. There should be no discernable pattern (such as a straight or curved line) to the dots.

2. About half the dots should appear in the top half of the graph.

Figure 8 demonstrates that virtually all observed DPA measurements are satisfactorily predicted by the method of the present invention. The horizontal scale shows values predicted by the method of the present invention. The vertical scale shows Cook's distances between observed DPA measurements and the predictions of those measurements by the method of the present invention Thus, the closer the dots are to a height of Cook = 0, the more accurate is the method of the present invention. Because Cook's distances tend to form a collection of numbers fitting an F-statistical distribution, the two outlier points at heights between 10 and 20 are not thought to be problematic in view of the large size of the sample.

It should be understood, of course, that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

Claims

What is claimed is:

1. A method of providing a diagnostic system for diagnosing osteoporosis and determining the severity of osteoporosis in a human or animal comprising the steps of:

a. measuring the severity of osteoporosis in a set of humans or animals with varying severity of osteoporosis by a standard method;

b. assigning the severity of disease a numerical value on a severity scale, the scale being from no disease to severe disease;

c. measuring the blood concentrations of a predetermined set of blood constituents in the set of humans or animals with varying severity of the disease; and

d. determining a numerical relationship between the set of blood concentrations and the severity of disease

2. The method of Claim 1, wherein the numerical relationship is calculated using multiple linear regression analysis.

3. The method of Claim 1, wherein the predetermined set of blood constituents comprises calcium, phosphate, total alkaline phosphatase, alkaline phosphatase isoenzyme, estradiol and progesterone.

4. The method of Claim 3, wherein the age of the human or animal is factored into the numerical relationship.

5. The method of Claim 3 wherein the alkaline phosphatase isoenzyme is selected from the group consisting of lymphocyte-derived alkaline phosphatase, liver alkaline phosphatase, bone alkaline phosphatase and liver alkaline phosphatase.

6. The method of Claim 1, wherein the standard method of measuring severity of osteoporosis is measuring bone density by radiographic absorptometry, quantitative computed tomography, dual photon absorptometry or direct measurement of bone density.

7. The method of Claim 6, wherein the standard method of measuring severity of osteoporosis is dual photon absorptometry.

8. A method of determining the diagnosing osteopenia in a human or animal comprising the steps of:

a. measuring the concentrations of a predetermined set of blood constituents in the blood of the human or animal,
b. determining a bone density coefficient using the numerical relationship of Claim 1; and
c. determining the severity of osteoporosis in the human or animal by comparing the bone density coefficient to the severity scale of Claim 1.

9. The method of Claim 8, wherein the predetermined set of blood constituents comprises calcium, phosphate, total alkaline phosphatase, alkaline phosphatase isoenzyme, estradiol and progesterone.

10. The method of Claim 9, wherein the alkaline phosphatase isoenzyme is selected from the group consisting of lymphocyte-derived alkaline phosphatase, liver alkaline phosphatase, bone alkaline phosphatase and liver alkaline phosphatase.

11. The method of Claim 8, wherein the age of the human or animal is factored into the numerical relationship.

```
**********************************
** Program : Calc.prg
** Author  : Terminal Solutions (JJK)
** Date    : 14 August 1992
** Purpose : QuiOs calculating program
**
**********************************
set decimal to 16
set fixed on
****** Set Coefficient information to valiables ******
store  0.000000000  to outnumb
store -1009.33  to constant
store -0.014786248   to c_age
store  424.462000000 to c_cal
store -66.3468       to c_cal2
store -0.025761569   to c_csage
store -0.019715699   to c_csphos2
store  0.027232306   to c_cstintes
store  0.000000263   to c_etwoimx2
store -12.0319       to c_phos
store  7.499334732   to c_phos2
store -2.220366330   to c_phos3
store  0.313008013   to c_phos4
store -0.016909117   to c_phos5
store  0.001369629   to c_prog2
store -0.000428831   to c_progpliv
store  0.001798351   to c_progtint
store  0.001841052   to c_tnetwoimx
store  0.007649946   to c_tntintes
store  0.002153604   to c_agephos
store  4.606320653   to c_cal3
store -0.119876531   to c_cal4
store  0.011786744   to c_talp
store -0.000170224   to c_talp2
store  0.000000706   to c_talp3
outnumb= (constant)
outnumb=outnumb +  (c_age * age)
outnumb=outnumb +  (c_cal * cal)
outnumb=outnumb +  (c_cal2 *  (cal * cal))
outnumb=outnumb +  (c_csage *  (cos(age)))
outnumb=outnumb +  (c_csphos2 *  (cos(phos * phos)))
outnumb=outnumb +  (c_cstintes *  (cos(tintes)))
outnumb=outnumb +  (c_etwoimx2 *  (etwoimx * etwoimx))
outnumb=outnumb +  (c_phos * phos)
outnumb=outnumb +  (c_phos2 *  (phos * phos))
outnumb=outnumb +  (c_phos3 *  (phos * phos * phos))
outnumb=outnumb +  (c_phos4 *  (phos * phos * phos * phos))
outnumb=outnumb +  (c_phos5 *  (phos * phos * phos * phos * phos))
outnumb=outnumb +  (c_prog2 *  (prog * prog))
outnumb=outnumb +  (c_progpliv *  (prog * pliv))
outnumb=outnumb +  (c_progtint *  (prog * tintes))
outnumb=outnumb +  (c_tnetwoimx *  (tan(etwoimx)))
outnumb=outnumb +  (c_tntintes *  (tan(tintes)))
outnumb=outnumb +  (c_agephos *  (age * phos))
outnumb=outnumb +  (c_cal3 *  (cal * cal * cal))
outnumb=outnumb +  (c_cal4 *  (cal * cal * cal * cal))
outnumb=outnumb +  (c_talp * talp)
outnumb=outnumb +  (c_talp2 * (talp * talp))
outnumb=outnumb +  (c_talp3 * (talp * talp * talp))
```

Figure 1

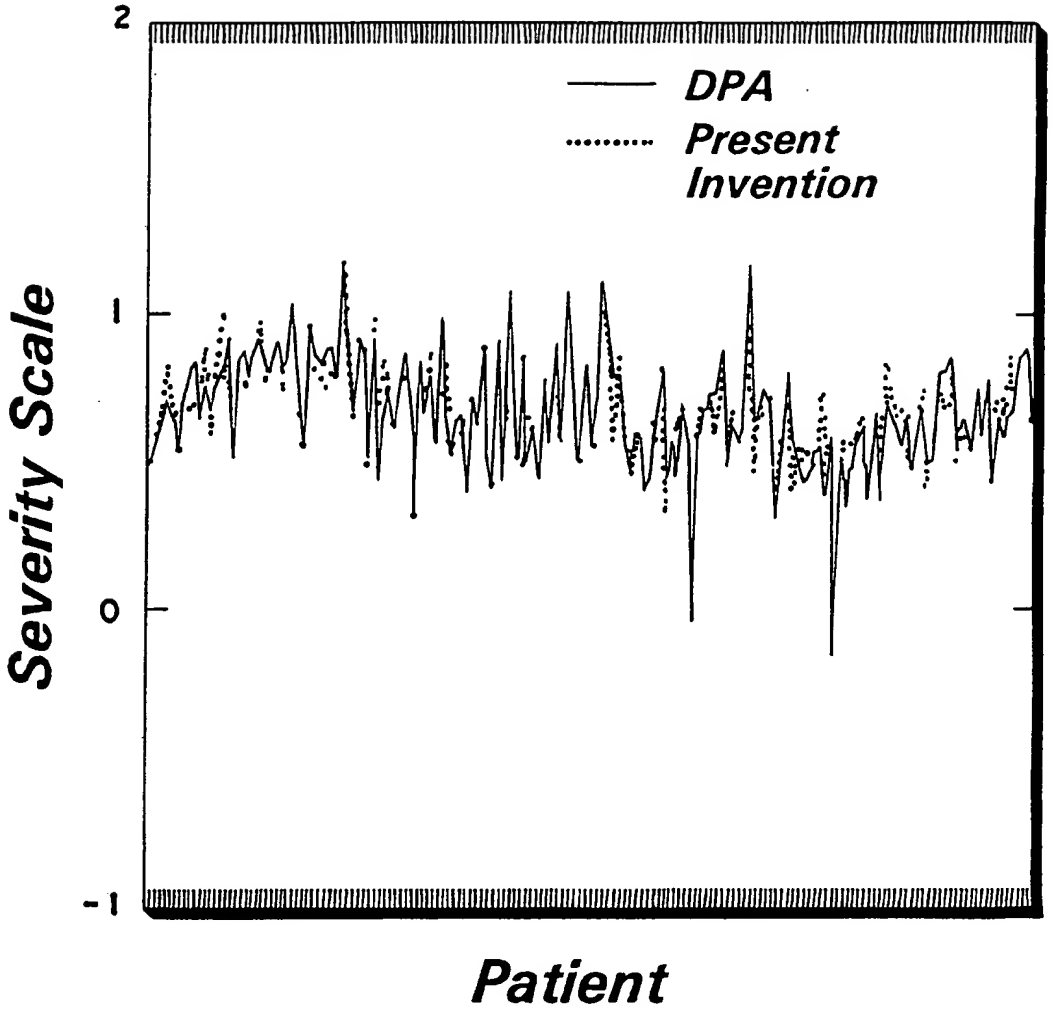

Fig. 2

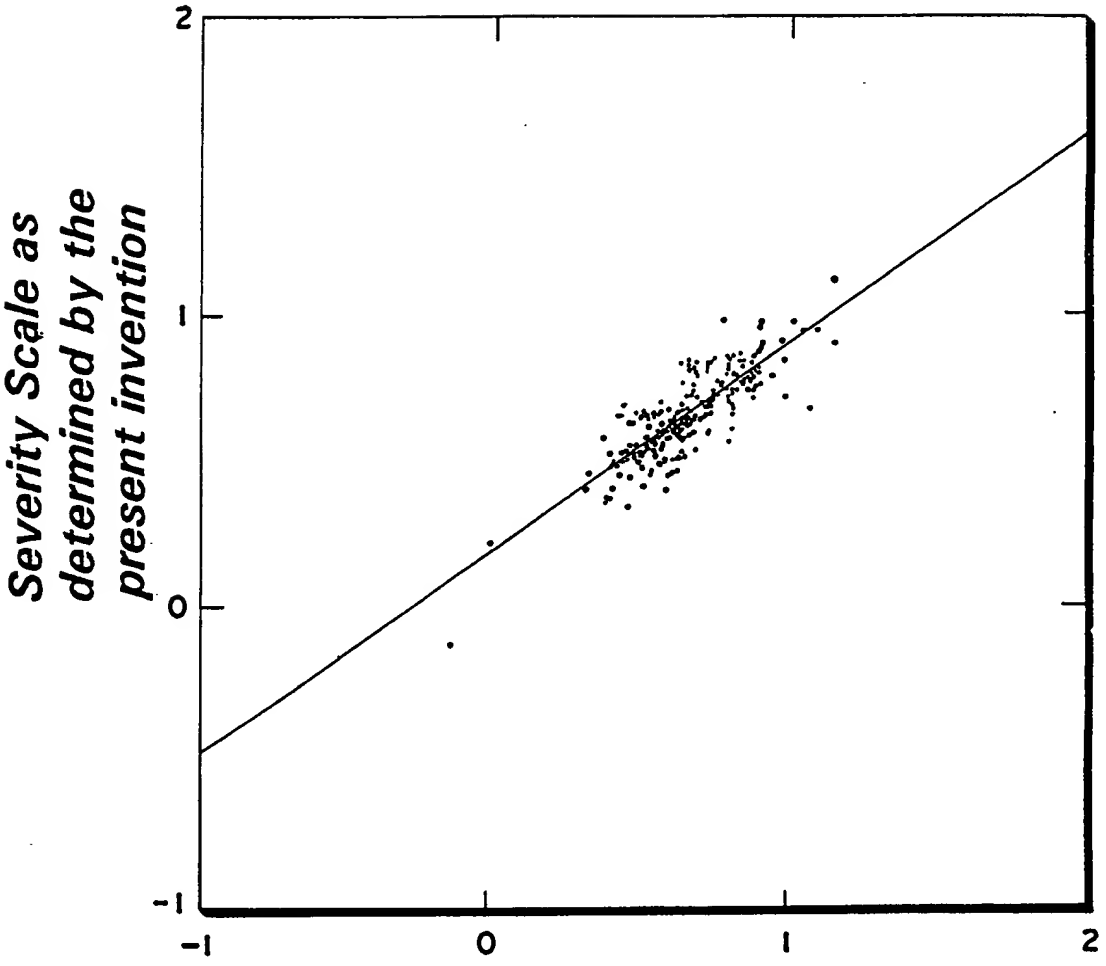
Severity Scale as determined by the present invention
2
1
0
-1
-1
0
1
2
Severity Scale as determined by DPA

Fig_3

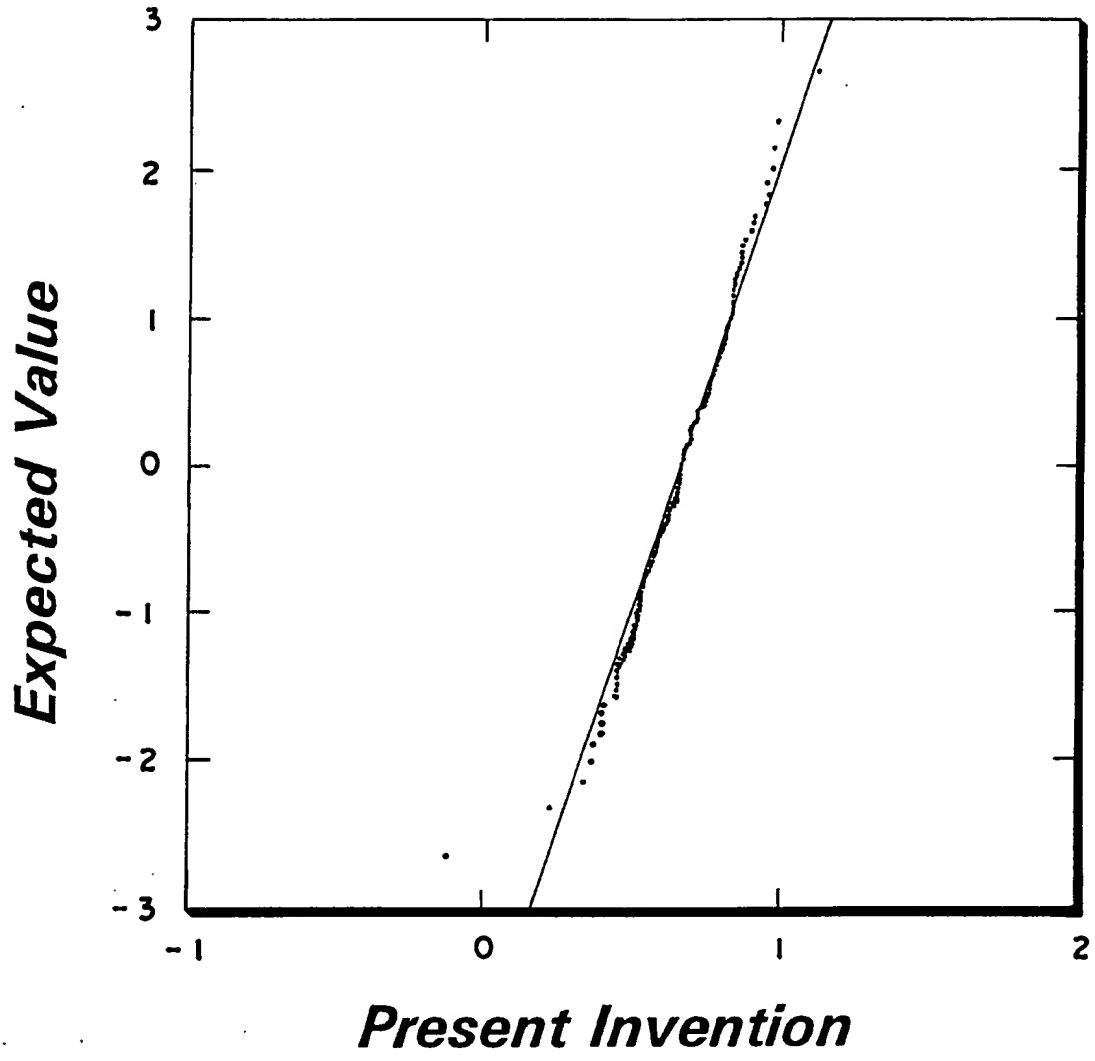

Fig. 4

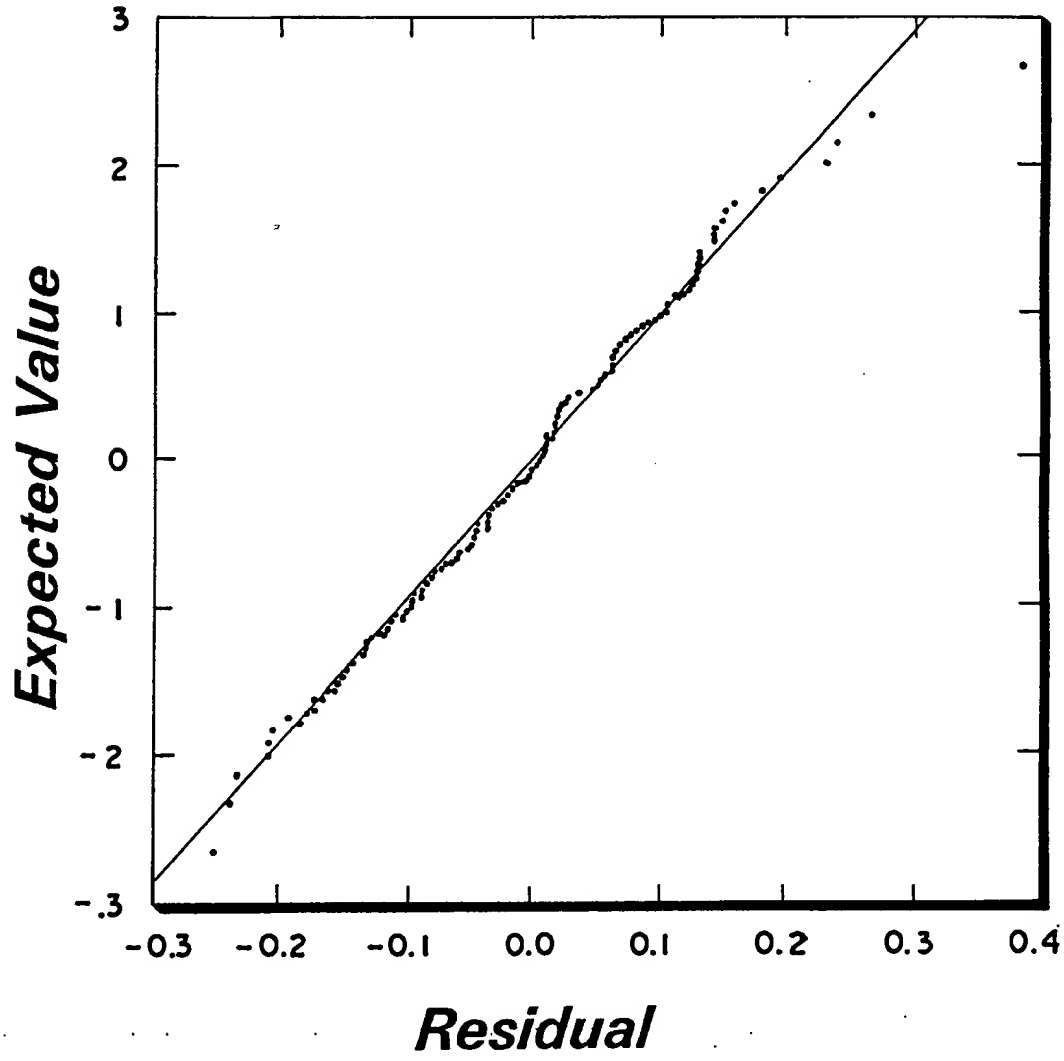

Fig_5

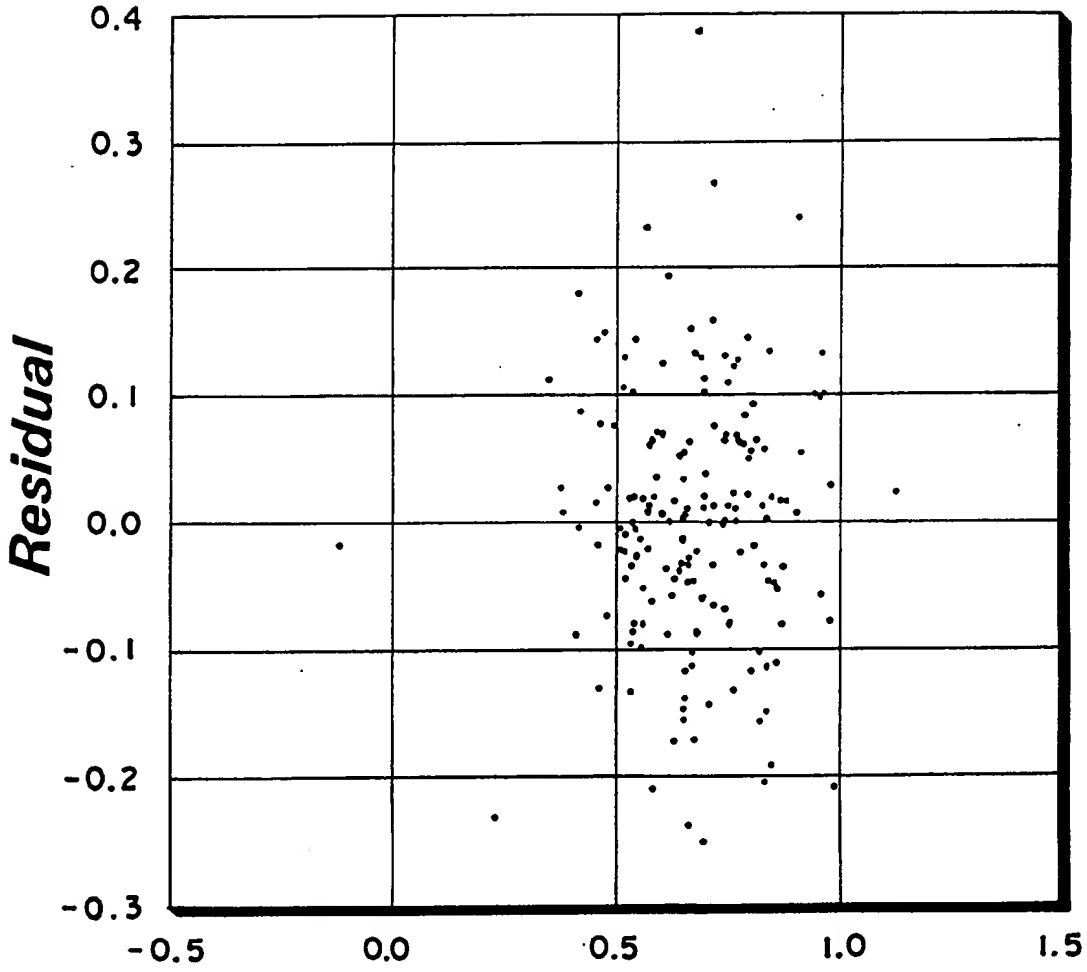
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
Residual
-0.5
0.0
0.5
1.0
1.5
Present Invention

Fig_6

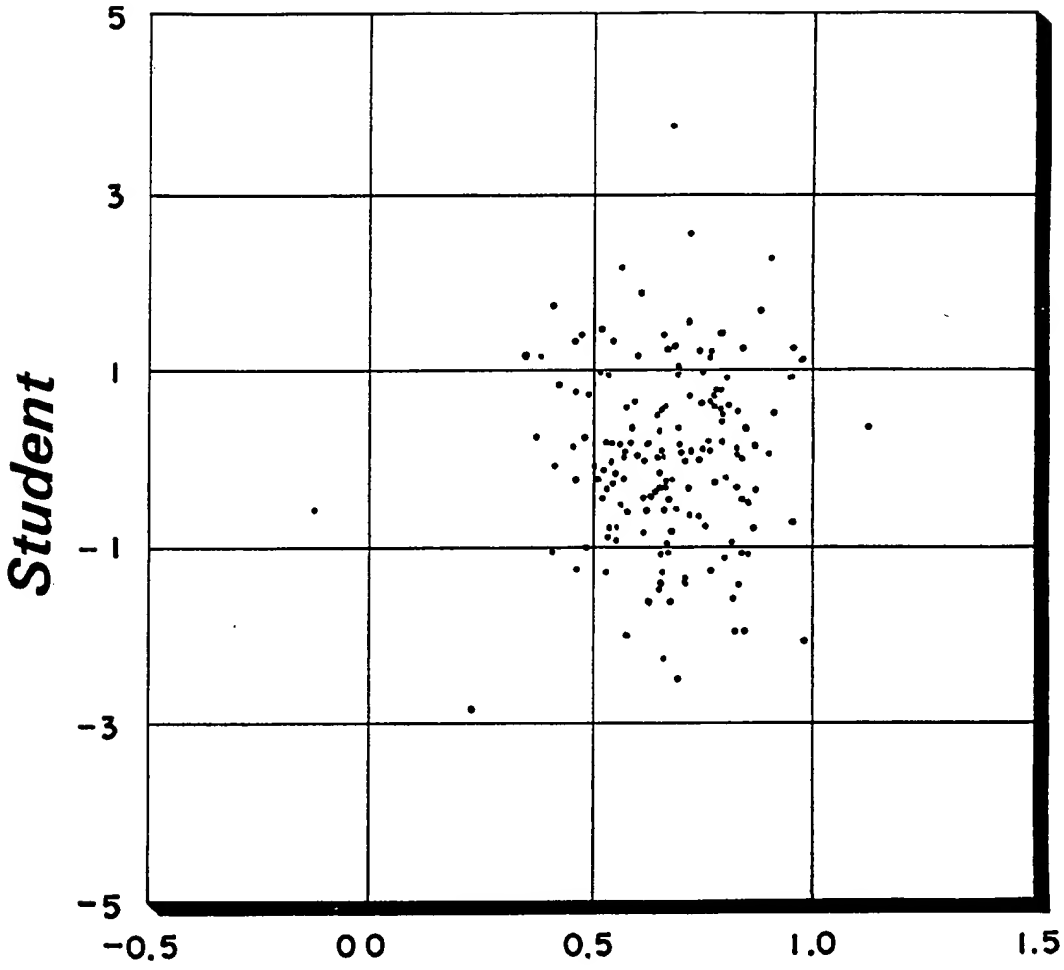

Fig 7

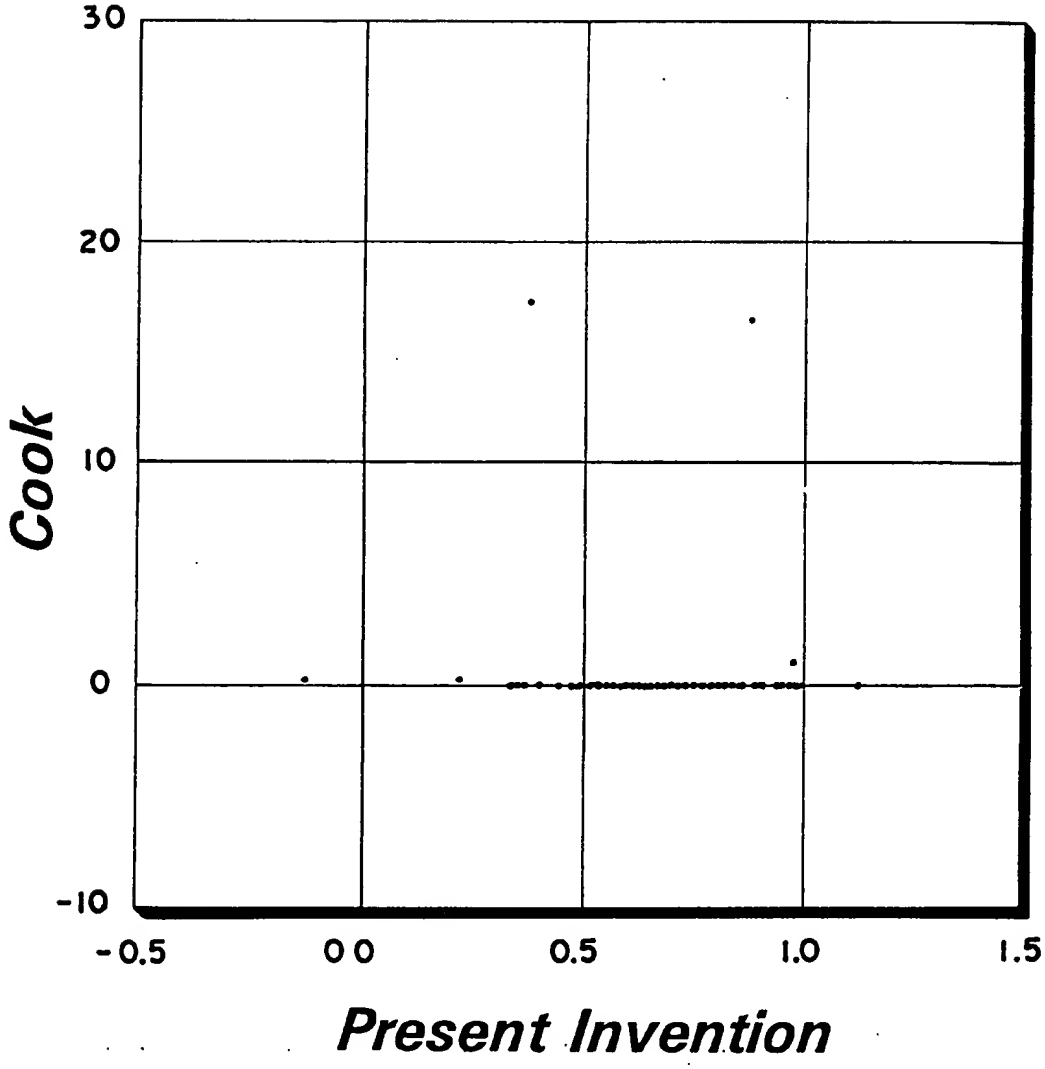

Fig. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10879

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12Q 1/42
US CL :435/21; 810; 436/808, 811
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/21, 810; 436/808, 811

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,635,643 (Brown) 13 January 1987. See entire document.	1-11
A	US, A, 4,740,364 (Hodgen) 26 April 1988. See entire document.	1-11
A	US, A, 4,857,456 (Urist) 15 August 1989. See entire document.	1-11
Y	Annals of Clinical and Laboratory Science, issued August (1987), Barnhill et al., "Osteoporosis: A possible Auutoimmune Etiology", Abstract of the 80th meeting of Associations of Clinical Scientists, See entire document.	1-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 MARCH 1993	25 MAR 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer ANITA VARMA SD Telephone No. (703) 308-0196

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